



Mitochondria and the Three Rs

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Mitochondrial toxicity is a predominant cause of late-phase drug attrition and a common cause of organ toxicity from medication already in clinical use. Investigations into the effect of xenobiotics on specific mitochondrial biochemical pathways may, therefore, help to improve our understanding and result in ways to prevent certain forms of organ toxicity.

FRAME is an organisation that encourages a realistic consideration of the ethical and scientific issues involved in the use of laboratory animals. The FRAME Alternative Laboratory (FAL) conducts research to advance the development and optimisation of non-animal alternative methods. Areas of research include the use of human hepatocytes, stellate cells and transcriptome profiling for risk assessment.

FRAME is interested in research that contributes to Reduction, Refinement or Replacement – the Three Rs – with Replacement of animal experiments being the ultimate goal. In this context, the use of mitochondrial studies as an approach to predict organ toxicity shows promise for toxicity testing and drug discovery.

How Mitochondria Contribute to the Three Rs

Cell models / Isolated organelles

These *in vitro* methods use human or animal cells or isolated mitochondria allowing a variety of endpoints to be measured. Mitochondrial functions that may be assessed include:

- Oxygen consumption
- Fatty acid oxidation (FAO)
- Proton-motive force (Δp)
- Reactive oxygen species (ROS)
- Membrane potential ($\Delta\Psi$)
- Mitochondrial DNA replication
- Changes in mitochondrial size
- Protein synthesis

Alterations in any of these parameters indicate that the compound being tested can affect mitochondria and has potential to cause toxicity.



Ex vivo / Non-invasive investigations

Experiments can also be carried out with *ex vivo* perfused organs (liver, heart), which can be subjected to histopathology and microscopic examination. To study mitochondrial function non-invasively, a few techniques can be used, for example, magnetic resonance spectroscopy (MRS) or positron emission tomography (PET). MRS allows the detection of metabolites that take part in metabolic pathways such as oxidative phosphorylation and anaerobic glycolysis while PET determines the kinetics of tracer molecules in selected tissues.



In vitro assays



Optimisation of high-throughput *in vitro* systems should improve the toxicological profiling of new molecules and reduce the number of drug candidates that fail after animal studies. Various cell-based platforms have been described to screen compounds for mitochondrial toxicity. One of such techniques consists of a set of non-radioactive capture immunoassays to identify molecules that affect oxidative phosphorylation (OXPHOS) complexes. A different microplate-based system measures the rates of oxygen consumption and acid efflux to

determine changes in bioenergetic states, which can be caused by xenobiotics. These methods have the advantages of yielding relatively fast results in a high-throughput format and of being able to narrow down the causes of mitochondrial toxicity. Therefore, the early detection of compounds with potential to cause mitochondrial impairment will contribute to a decrease in the number of animals used in drug development and testing. Research into the role of mitochondria in drug-induced toxicity should also assist with the development of more predictive pre-clinical models.

